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Diurnal and circadian expression profiles of glycerolipid biosynthetic genes in *Arabidopsis*

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Glycerolipid composition in plant membranes oscillates in response to diurnal change. However, its functional significance remained unclear. A recent discovery that *Arabidopsis* florigen FT binds diurnally oscillating phosphatidylcholine molecules to promote flowering suggests that diurnal oscillation of glycerolipid composition is an important input in flowering time control. Taking advantage of public microarray data, we globally analyzed the expression pattern of glycerolipid biosynthetic genes in *Arabidopsis* under long-day, short-day, and continuous light conditions. The results revealed that 12 genes associated with glycerolipid metabolism showed significant oscillatory profiles. Interestingly, expression of most of these genes followed circadian profiles, suggesting that glycerolipid biosynthesis is partially under clock regulation. The oscillating expression profile of one representative gene, *PECT1,* was analyzed in detail. Expression of *PECT1* showed a circadian pattern highly correlated with that of the clock-regulated gene *GIGANTEA*. Thus, our study suggests that a considerable number of glycerolipid biosynthetic genes are under circadian control.

Glycerolipids are primary structural components of biological membranes and serve as a form of energy and carbon storage. Therefore, glycerolipids tend to be recognized as fairly inert end products of metabolic pathways. However, the composition of glycerolipids, including the relative amounts of different lipid classes as well as of the molecular species of fatty acyl moieties esterified to the glycerol backbone, fluctuates in response to environmental factors.[1] One important factor is light, as it was shown that light stimulates the activity of some of the lipid biosynthetic enzymes.[1] For example, the activity of acetyl-CoA carboxylase, which catalyzes the initial step of fatty acid biosynthesis, depends on light, so that de novo synthesis of fatty acid exclusively takes places during light period.[2] Moreover, light exposure stimulates the activity of monogalactosyldiacylglycerol (MGDG) synthase in cucumber.[3] MGDG production is a prerequisite for the establishment of photosynthetic membranes because MGDG is an essential component of the thylakoids. Diurnal fluctuations of fatty acid composition of glycerolipids in spinach leaves are associated with elevated levels of polyunsaturated fatty acids, such as linoleic acid (18:2) or linolenic acid (18:3), during the dark period.[4] By contrast, saturated fatty acid species become dominant during the light period, showing clear diurnal change of fatty acid unsaturation level in the composition of phosphatidylcholine (PC) as well as total glycerolipids.[4] This profile was confirmed in *Arabidopsis* leaves,[5] and extensive lipidomic profiling revealed that levels of phosphatidic acid (PA) and phosphatidylserine (PS) increase during the dark period.[6] These data indicate that glycerolipid composition is actively regulated during the diurnal cycle. However, the mechanisms of how these changes affect plant growth and development are poorly understood. A recent study revealed that the *Arabidopsis* florigen FLOWERING LOCUS T (FT) protein specifically binds to PC in vitro, and the increase in PC at the shoot apex accelerates flowering in vivo.[7] Interestingly, FT does not preferably bind to PC containing polyunsaturated fatty acids, which are dominant molecular species during the dark period, but it strongly binds to PC molecules enriched in saturated fatty acids. This specificity may be important in regulating flowering time, because overexpression of *FATTY ACID DESATURASE3* (*FAD3*) which results in the increase in night-dominant PC (polyunsaturated) species in the daytime delays the flowering time even in the presence of active FT protein.[7] This observation suggests that diurnal oscillation of PC molecular species alters the levels of PC-bound FT affecting flowering induction.

Because the regulation of diurnal profiles of glycerolipid amounts is so precise, we wondered whether diurnal changes of glycerolipid biosynthesis are also controlled at the transcriptional level. We therefore explored gene expression profiles of

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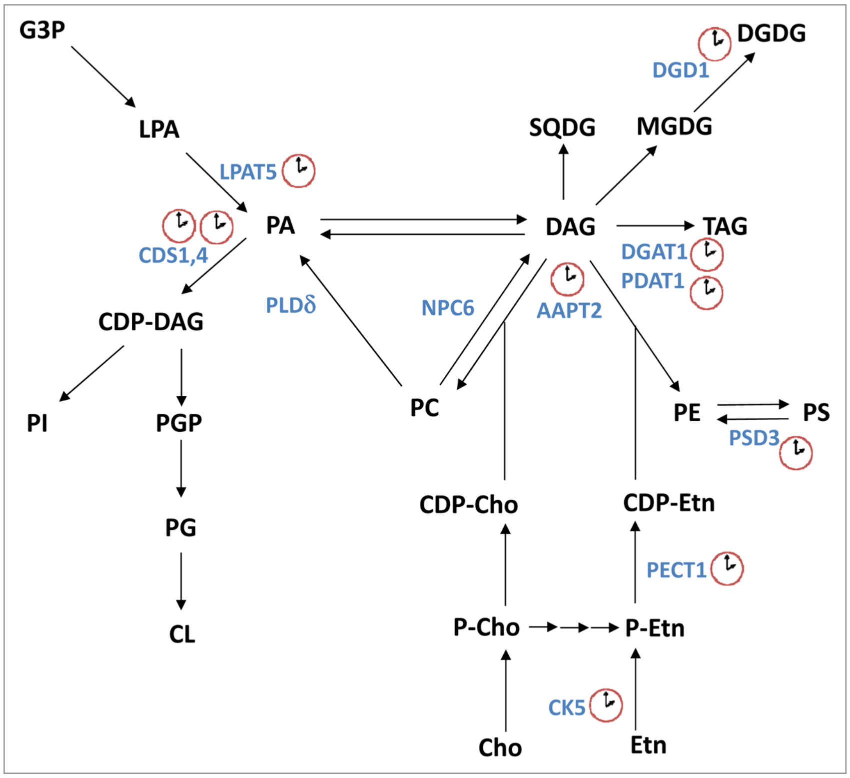

Figure 1. Glycerolipid metabolic map in *Arabidopsis* and reaction steps associated with diurnal or circadian oscillation of gene expression. Genes indicated in blue letters show a significant diurnal profile, and those with clock icons reveal circadian profiles of gene expression. The web-based tool "Diurnal" (http://diurnal.mocklerlab.org/) was used to acquire diurnal/circadian gene expression profiles of glycerolipid biosynthetic genes from public microarray data. Gene expression profiles of long-day (LD; 16h light and 8h dark), short-day (SD; 8h light and 16h dark) and continuous light (LL; continuous light) conditions were compared through consecutive 48-h intervals available at this database. Significance of oscillation was determined by Pearson's correlation of 0.8 or greater, which is given in this web tool. Abbreviations are as follows for compounds (CDP-Cho, cytidine diphosphocholine; CDP-Etn, cytidine diphosphoethanolamine; CDP-DAG, cytidine diphosphodiacylglycerol; Cho, choline; CL, cardiolipin; DAG, *sn*-1,2-diacylglycerol; DGDG, digalactosyldiacylglycerol; Etn, ethanolamine; G3P, glycerol 3-phosphate; LPA, lysophosphatidic acid; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; P-Cho, phosphorylcholine; PE, phosphatidylethanolamine; P-Etn, phosphorylethanolamine; PG, phosphatidylglycerol; PGP, phosphatidylglycerol phosphate; PI, phosphatidylinositol; PS, phosphatidylserine; SQDG, sulfoquinovosyldiacylglycerol) and enzymes (AAPT, aminoalcohol aminophosphotransferase; CDS, CDP-DAG synthase; CK, Cho kinase; DGAT, DAG acyltransferase; DGD, DGDG synthase; LPAT, LPA acyltransferase; NPC, non-specific phospholipase C; PDAT, phospholipid:DAG acyltransferase; PECT, CTP:phosphorylethanolamine cytidylyltransferase; PLD, phospholipase D; PSD, PS decarboxylase).

glycerolipid biosynthetic genes by taking advantage of public microarray data through a web-based tool "Diurnal" (http://diurnal.mocklerlab.org/). Here, we examined whether each of the glycerolipid biosynthetic genes shows diurnal or circadian profiles of expression in consecutive 48-h intervals available at this database. Diurnal change was recognized if a periodical profile was observed but altered between short day (SD; 8h light and 16h dark) condition and long day (LD; 16h light and 8h dark) condition. Circadian change was acknowledged if the periodic profile was unchanged between LD and SD, and such profile was observed under continuous light (LL; 24h light) condition. As demonstrated in **Figure 1**, 12 genes which potentially catalyze 10 reaction steps of glycerolipid metabolism showed significant oscillatory profiles. Moreover, 9 of them showed circadian profiles of gene expression, suggesting that the majority of these glycerolipid biosynthetic genes rely on circadian clock rather than diurnal environmental cues. To verify this profile experimentally, we compared the expression pattern of *CTP:phosphorylethanolamine cytidylyltransferase1* (*PECT1*) between LD and SD, as an example for the genes listed in **Figure 1**, because *PECT1* is one of the best characterized genes for its critical role in phospholipid metabolism and multiple aspects of plant development.[8,9] As shown in **Figure 2**, the expression of *PECT1* showed transient peaks at ZT8 and ZT32, repeatedly both in SD and LD conditions, and these peaks correlated well with those of *GIGANTIA* (*GI*), a representative clock-regulated gene.[11] FT is induced at LD but not in SD condition even though *PECT1* shows a constant oscillating expression pattern. This result confirmed circadian-regulated expression of *PECT1*.

What is the possible functional significance in circadian regulation of glycerolipid composition? Diurnal changes in the composition of thylakoid lipids, including MGDG and phosphatidylglycerol (PG), may be associated with the specific lipid requirements of the photosynthetic complexes. Structural analyses of the photosystems I and II revealed the presence of

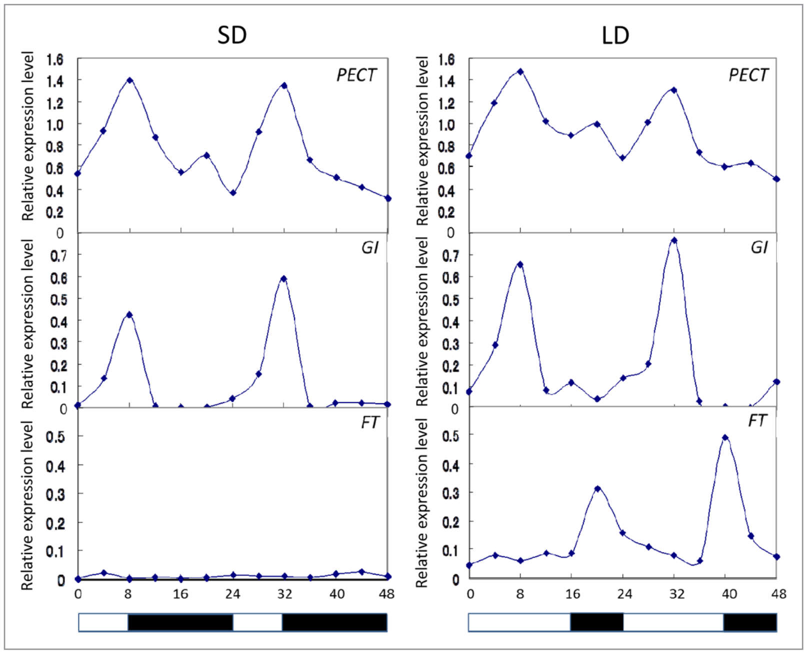

Figure 2. Circadian expression profile of *PECT1*. Plants were grown for 14 d under SD condition under a light intensity of 150 mmol/m^2/s at 21 °C. Plants were then shifted to LD or SD for 24h and time course sampling was performed for a subsequent 48h interval as previously described.[10] Total RNA was extracted using the RNAeasy kit (Qiagen) and treated with DNaseI (Ambion). cDNA synthesis was performed with SuperScript II (Invitrogen) from 1–3 µg of DNA-free RNA and diluted to a final volume of 200 µL with water. Three µL of diluted cDNA were used for each quantitative RT-PCR reaction. qRT-PCR reactions were prepared using the iQ SYBR Green supermix (Bio-Rad) and performed in a LightCycler 480 thermal cycler (Roche). *PEX4* (At5g25760) was used to normalize the expression of the genes investigated. *X*-axes are shown in ZT time and values on the *y*-axes indicate gene expression levels relative to *PEX4*. A representative set of 3 biological replicates is shown. The primer sequences used for qRT-PCR experiments are; *PECT1* (Fw TATGCACTTG CTAAGAAGGC TG /Rv TTGCAGAGAG GAACGACTAT GA), *GI* (Fw CTGTTCAGAC GTTCAAAGGC /Rv TGGTTTCCTC TTGGATTCAT), *FT* (Fw TGGTGACTGA TATCCCTGCT /Rv ACCCTGGTGC ATACACTGTT) and *PEX4* (Fw TTACG AAGGC GGTGTTTTTC /Rv GGCGAGGCGT GTATACATTT).

galactolipids (MGDG, DGDG), PG and of the sulfolipid sulfoquinovosyldiacylglycerol (SQDG), closely bound to the polypeptide chains. In addition, lipids in the bilayer play an important role for embedding the complexes into the thylakoid membrane. Therefore, it is conceivable that diurnal changes in acyl composition of thylakoid lipids might have an impact on photosynthetic activity. Furthermore, it is known that central carbon metabolism is under diurnal and circadian control. Expression of genes involved in the breakdown of starch show coordinated diurnal changes partially driven by the clock.[12-15] Indeed, the clock system anticipates the length of the dark period and sets the rate of degradation so that starch is almost exhausted at dawn.[16] Moreover, such a carbon distribution was shown to be involved in the floral transition.[17] Based on our analysis, it is likely that triacylglycerol (TAG) levels and/or composition show circadian changes, because *acyl-CoA:diacylglycerol acyltransferase1* (*DGAT1*) and *phospholipid:diacylglycerol acyltransferase1* (*PDAT1*), which provide the predominant capacity for TAG production,[18] show circadian profiles of gene expression (**Fig. 1**). In mammals, clock genes are involved in the homeostasis of plasma lipids,[19] and circadian rhythms function in adipocytes during the development of obesity.[20] Apart from the carbon distribution, the circadian profiles may regulate lipid signaling. Glycerolipids are important precursors of oxylipin signals, such as jasmonic acid (JA) and green leaf volatiles for defense responses and plant-microbe interactions. Indeed, *MYC2*, a key transcriptional regulator for JA signaling is negatively regulated through the direct interaction with the circadian-clock component *TIME FOR COFFEE* (*TIC*), so that JA signaling is directly influenced by a circadian oscillator.[21] Furthermore, this circadian fluctuation of JA levels and the resulting defense response are correlated with the circadian activities of insects.[22]

In conclusion, we revealed that a number of glycerolipid biosynthetic genes showed circadian patterns of expression in *Arabidopsis*, including *PECT1*, which shows highly correlated expression patterns with the primary clock-regulated gene *GI*. Functional studies are anticipated to unravel roles of circadian regulation of lipids in plant development and regulation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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